

· 论 著 ·

脐血造血干细胞低温保存方法探讨

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摘要 为使脐血有更广阔的应用前景及建立脐血造血干细胞(CBSC)库,笔者探讨了脐血低温保存的有效方法。结果显示:CBSC 4℃低温保存以 2~3d 为宜;液氮低温保存是一种有效的 CBSC 保存方法,回收率高,较长时间保存,回收率不受影响;-80℃冰箱保存法具有与液氮相同的低温保存效果;且简单、经济、方便和实用,无需程序降温仪,便于大规模开展应用和建立 CBSC 库;脐全血保存可以避免分离过程 CBSC 的丢失,保存效果不受影响。

关键词 脐带血 造血干细胞 低温保存

脐血造血干细胞(CBSC)移植临床已获得成功,其更广阔的应用前景,有赖于 CBSC 库的建立和无关供者 CBSC 移植的开展。为此,探讨有效的 CBSC 低温保存方法和一些影响低温保存效果的因素极为重要。

1 材料方法

1.1 标本来源 正常孕妇顺产的新生儿脐血,ACD 抗凝。

1.2 联合低温保护剂 低温保存前 30min 配制:12%羟乙基淀粉(HES)+10%AB 型血清+10%二甲基亚砷(DMSO)+68%RMPI1640 液,充分混匀后,4℃保存备用。

1.3 脐血低温保存方法

1.3.1 4℃保存法 新鲜脐血放入 4℃冰箱保存,第 1、2、3、5d 时取出,检测有关指标。

1.3.2 深低温保存法 新鲜脐血分为 2 等份,一份全血保存,另一份经 1640 液等倍稀释后,2000rpm 离心 20min,收集富含白细胞的层(白层)低温保存;两者的细胞浓度均调整至 $10.0 \times 10^6/\text{ml}$ 。两份脐血(全血和白层)和联合低温保护剂分别于 4℃预冷 20~30min,在冷冻袋中 1:1 混匀,经 4℃平衡 15~20min,将全血和白层各分成 3 等份,用 3 种不同方法作低温保存:脐血直接放入 -80℃低温冰箱中保存;经程序降温仪逐步冷冻至 -80℃后,放入液氮液相温

区保存;经程序降温仪逐步冷冻至 -80℃后,放入 -80℃低温冰箱中保存。复温均采用快速复温法,42℃水浴复温,100s 内解冻完毕。

1.4 观测指标 分别检测低温前后的有核细胞计数(NC)、单个核细胞计数(MNC)、台盼蓝拒染率(拒染率)和 CFU-GM,并计算低温回收率。

2 结果

2.1 脐血 4℃保存 CBSC 的活力变化 脐血经 4℃保存 1~5d, CBSC 活力变化见表 1。结果显示,4℃保存第 1、2d 对造血细胞活性无明显影响,细胞数、CFU-GM 产率和拒染率均保持了较高水平;第 3d 时,CFU-GM 产率减少一半;5d 后几乎无 CFU-GM 生长。

表1 脐带血全血造血细胞 4℃保存后的活性(n=11)

保存时间 (d)	NC ($\times 10^9/\text{L}$)	MNC ($\times 10^8/\text{L}$)	拒染率 (%)	CFU-GM 产率 ($/2 \times 10^5$)
1	16.6 $\pm$ 3.2	6.0 $\pm$ 2.4	100 $\pm$ 0	261 $\pm$ 24.5
2	16.7 $\pm$ 4.1	5.3 $\pm$ 0.8	91 $\pm$ 9	197 $\pm$ 50.2
3	12.1 $\pm$ 1.6	5.0 $\pm$ 1.1	83 $\pm$ 10	138 $\pm$ 54.5
5	11.1 $\pm$ 2.3	4.8 $\pm$ 1.8	72 $\pm$ 12	29 $\pm$ 32.1*

* 5 例的结果

2.2 CBSC 深低温保存效果观察

2.2.1 3 种深低温保存方法效果比较 应用 3 种不同的深低温方法保存脐全血与白层 5 例,时间为 1 个月。析因分析统计结果显示,低温保存的脐全血与白层之间(P_1),3 种不同的低温

保存法之间(P_2)、全血或白层与 -80°C 冰箱或液氮间6种不同的组合比较时,NC、MNC、拒

染率和CFU-GM 4项指标的回收率均无显著差异,低温保存效果相同(表2)。

表2 3种低温保存法的回收率(%)比较($\bar{x} \pm s$)

	全 血			白 层			P_1	P_2	P_3
	方法1	方法2	方法3	方法1	方法2	方法3			
NC	90.8 $\pm$ 9.6	92.5 $\pm$ 9.1	89.3 $\pm$ 5.9	87.4 $\pm$ 10.1	82.5 $\pm$ 19.4	81.7 $\pm$ 17.5	0.15	0.82	0.85
MNC	96.4 $\pm$ 11.3	97.9 $\pm$ 14.8	95.5 $\pm$ 17.8	91.8 $\pm$ 15.6	90.1 $\pm$ 19.9	87.0 $\pm$ 21.7	0.29	0.91	0.96
拒染率	74.5 $\pm$ 8.1	81.2 $\pm$ 4.2	75.6 $\pm$ 5.4	75.9 $\pm$ 3.5	83.0 $\pm$ 5.9	73.1 $\pm$ 9.6	0.87	0.02	0.74
CFU-GM	62.0 $\pm$ 9.5	64.4 $\pm$ 9.1	61.3 $\pm$ 6.4	61.7 $\pm$ 10.7	60.5 $\pm$ 16.0	58.1 $\pm$ 11.5	0.54	0.84	0.93

注: P_1 全血与白层比较; P_2 不同保存方法间比较; P_3 交互作用

2.2.2 CBSC液氮保存回收率的动态变化 9例脐血分别作全血和白层的液氮保存(图1~4)。析因分析显示,各时点间回收率以及较长时间液氮保存的脐全血和白层的保存效果均无显著差异。

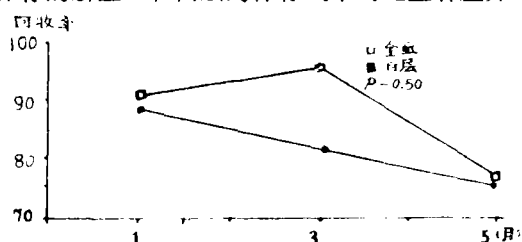


图1 液氮低温保存 NC 回收率的动态变化

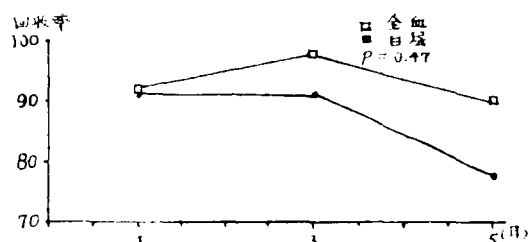


图2 液氮低温保存 MNC 回收率的动态变化

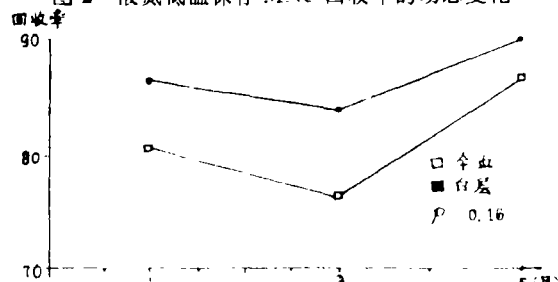


图3 液氮低温保存台盼蓝拒染率的动态变化

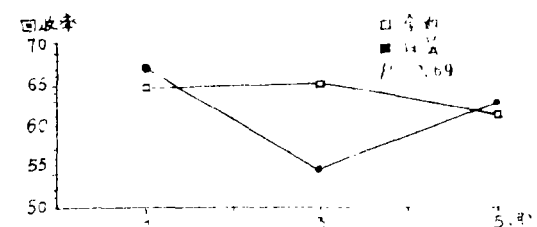


图4 液氮低温保存 CFU-GM 回收率的动态变化

2.2.3 CBSC -80°C 保存回收率的动态变化

15例脐血同样分别作全血和白层 -80°C 直接低温保存(图5~8)。统计结果表明,脐血 -80°C 保存6个月,4项指标回收率不受影响;动态观察还显示,脐全血保存回收率略高于白层,统计处理证实全血 MNC 回收率明显高于白层($P=0.03$)。

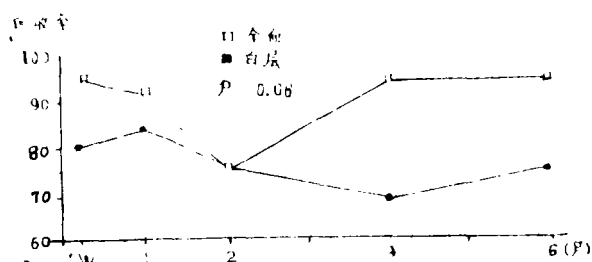


图5 -80°C 低温保存 NC 回收率的动态变化

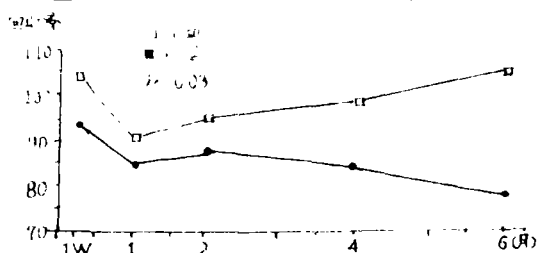


图6 -80°C 低温保存 MNC 回收率的动态变化

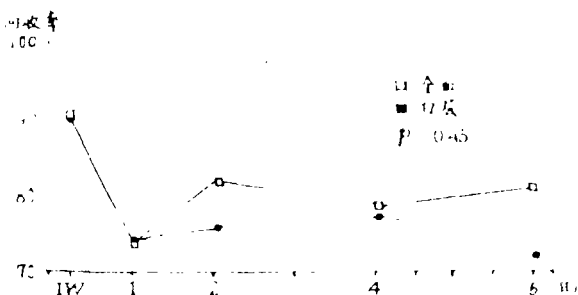


图7 -80°C 低温保存台盼蓝拒染回收率的动态变化



图8 -80℃低温保存CFU-GM回收率的动态变化

3 讨论

4℃冰箱保存3d的骨髓自体移植已有文献报道^[1],该法设备简单方便,样本不需处理。笔者应用4℃保存脐血2d内CFU-GM产率稳定,3d后产率大大降低,说明4℃冰箱保存CBSC以<2~3d为宜,临床上应用4℃保存的脐血作干细胞移植或输注时,亦应在3d内使用,且要避免病原菌的污染。

应用液氮保存CBSC,4项指标的回收率分别为NC:(82.0±6.9)%,MNC:(87.2±7.2)%,拒染率:(86.7±6.3)%,CFU-GM:(60.5±7.1)%,与液氮保存的骨髓和外周血干细胞回收率基本相同^[2,3],也与其他作者报道的脐血CFU-GM低温回收率结果一致^[4]。笔者认为液氮保存CBSC可获得良好的效果,且较长时间保存效果稳定。

应用-80℃冰箱直接保存CBSC,并与液氮保存和程序降温后-80℃冰箱保存效果比较,结果证明-80℃冰箱直接法具有后两者相同的低温回收率,保存效果确实、可靠,本法与其他作者-80℃冰箱保存的骨髓和外周血干细胞结果一致^[5,6]。-80℃冰箱保存法简单、经济、方便,避免了程序降温和液氮保存法的繁琐与

保存空间的限制,明显降低了成本,可望用于脐带血的大量低温保存。半年来的观察结果便证明,-80℃冰箱可用于较长期地保存CBSC和建立脐血细胞库。

脐血容量有限,为满足移植需要,避免NC分离时CBSC丢失极为重要^[4]。为此,笔者探讨了脐全血低温保存的可行性,经与传统离心白层保存效果比较证实,脐全血液氮与-80℃冰箱低温保存均可获得良好保存效果,且较长时间保存回收率稳定,故而,供移植使用的脐血和建立脐血细胞库时,应以全血保存为宜。

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ENGLISH ABSTRACTS OF ORIGINAL ARTICLES

Approaching the Method of Cryopreserving Hemopoietic Stem Cells in Cord Blood

Zhu Weiguo, et al

(Nan Fang Hospital of First Military Medical University)

For the purpose of opening up the future of more widespread application of cord blood, and also for the purpose of setting up the bank of hemopoietic stem cells in cord blood, an effective method of cryopreserving the cord blood was approached, indicating that it was appropriate that the time of cryopreserving the hemopoietic stem cells in cord blood at 4°C should be 2~3 days; the storage of hemopoietic stem cells in cord blood with liquid nitrogen was an effective method of cryopreserving the hemopoietic stem cells in cord blood, with high recovery rate, and longterm cryopreservation without any change in the recovery rate; the effect of storing the hemopoietic stem cells in freezer at -80°C was similar to that of cryopreserving them with liquid nitrogen, and the former was simple, economic, convenient and practical, and needed no any programmed temperature-dropping apparatus. At the same time, it is convenient to using the stem cells in large scale and to setting up the bank of them. Preserving the whole cord blood can avoid the loss of hemopoietic stem cells in cord blood during the centrifuging process, with the effect of preservation being unchanged.

Key words: Cord blood; Hemopoietic stem cell; Cryopreservation

(Original article on page 58)

Analysis of the Biological Activity of and the Residual SD in the Prothrombin Complex Concentrate after Inactivation of Virus by SD

Yu Rong, et al

(Institute of Blood Transfusion, Chinese Academy of Medical Sciences)

The biological activity recovery rate of and the residual SD in the 6 batches of prothrombin complex concentrate after inactivation of virus by solvent-detergent was investigated. The average recovery rate of PE activity of prothrombin compound was 87.83%, the residual Tween 80 was < 40µg/ml and TNBP was < 10µg/ml. The TNBP in prothrombin complex concentrate was emphatically approached by gas chromatography, and the method of determination of TNBP in prothrombin complex concentrate using n-octadecane as internal standard, was set up. The linear relationship was good at the concentration ranging from 2 to 70µg/ml. The minimal concentration which could be detected was 1µg/ml. The recovery rate was 92.6%~95.5%.

Key words: Prothrombin complex concentrate Solvent-detergent (SD); Biological activity; Residual SD; Gas chromatography

(Original article on page 61)

Observation on the Hepatic Histology and Serology Changes in HCV-infected Blood Donors

Shi Xuanling, et al

(Institute of Blood Transfusion, Chinese Academy of Medical Sciences)

Seven asymptomatic blood donors, in whom the infection with HCV was verified, were selected. The time of their infection with HCV was one and a half years. And the research on the hepatic histology and serology indexes of the donors was performed. The liver tissue samples gotten by needle biopsy of liver were examined under the common microscope and electron microscope, thereby the abnormal hepatic histology manifestation was found in all the donors. The samples of seven cases have shown the pathological changes characteristic of early stage of viral hepatitis under microscope. The result of examination under electron microscope confirmed that the infected donors were located in early stage of infection, and showed the low grade hepatic histological lesion. The serological examination showed that all the seven cases had normal ALT level, and most of them were HCV RNA-positive and had genotype-1. It is necessary to conduct follow-up observation on the relationship between the changes of serological indexes and the hepatic lesion.

Key words: HCV-infection; Blood donor; Liver tissue; Serology.

(Original article on page 64)